Proposed Project: Implementation of the Isolated Perfused Liver to Study Nicotine Metabolism and Metabolic Interactions

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1. Proposed Research Related to Nicotine

A number of *in vitro* studies have been performed to determine the role of the liver and its different subcellular fractions in the metabolism of nicotine. In general, the results of these studies indicate the hepatic metabolism of nicotine is complex in that a number of metabolites have been identified and at least two subcellular fractions of the hepatocyte participate in these processes. As a result of these complexities, it has been difficult to study hepatic nicotine metabolism under conditions consistent with those thought to prevail *in vivo*. We have recently acquired the knowledge and techniques necessary to study the hepatic metabolism of a number of different agents by using isolated perfused liver preparations from either rats or mice. This system is especially valuable since it is possible to control such factors as hepatic blood flow and tissue oxygen concentrations and gradients. Further, by using the isolated perfused liver system, it is possible to collect large volumes of perfusates from which minor metabolites can be isolated and characterized. Hepatic biliary excretion of a drug or its metabolites can also be monitored by cannulating and collecting from the common bile duct.

We feel that the isolated perfused liver system is especially suited to continue the study of nicotine metabolism in our laboratories. First, selective and sensitive analytical techniques for quantitating nicotine and its metabolites are routinely used in our laboratories. Secondly, we have developed methods to chronically treat animals with cigarette smoke which would allow us to study the effect of cigarette smoke on nicotine metabolism in isolated perfused livers surgically prepared from rats or mice subjected to cigarette smoke. In summary, given our current level of expertise in studying nicotine metabolism, we feel that implementation of the isolated perfused rat or mouse liver preparation would yield valuable information concerning the metabolic and environmental factors influencing nicotine disposition.

2. Proposed Research Plans

a) Our initial studies will evaluate basal rates of nicotine metabolism in isolated perfused livers from rats and mice. We will employ an open perfusion system as opposed to the closed recirculating system used by some investigators. An open system will allow us to collect and analyze large volumes of the perfusate exiting the liver for concentrations of nicotine, cotinine, nicotine N-oxide, and any other metabolites which can be detected. It would

also be possible to add metabolites such as nornicotine or cotinine directly to the perfusate, in the absence of nicotine. This would enable us to add large enough quantities of these metabolites to the perfusate in order to generate concentrations of secondary minor metabolites that are easily detectable and quantifiable. Thus, the perfused liver preparation could be used as a tool to study both major and minor metabolites of nicotine. Once the system has been validated, we will also attempt to determine the concentration of these metabolites in bile collected from cannules placed in the common bile duct.

b) Very little is known about the hepatic metabolism of d-nicotine as compared to the naturally occurring l-nicotine. The perfused liver preparation would be an ideal system for this characterization. Thus, upon completion of part 2a, we would determine metabolic rates and profiles of d-nicotine using the isolated perfused liver.

c) Once we have established the basal metabolic rates and profiles outlined in 2a, identical studies will be performed on perfused livers from animals chronically exposed to cigarette smoke.

d) The isolated perfused liver preparation is novel for studying the interactions of nicotine with the metabolism of a number of different therapeutic agents. Initially, we will use the liver preparation to assess the effect of nicotine on general pathways of drug metabolism such as hydroxylation and dealkylation, since we currently have expertise in measuring substrates and products for these metabolic pathways. If interesting effects are observed, the studies could be extended to determine the effect of nicotine on therapeutic agents metabolized by these same pathways. This same approach could also be used to determine the effect of chronic cigarette smoking on the metabolism of therapeutically important agents. Although specific reports have appeared in the literature concerning the effect of chronic cigarette smoking on in vivo drug metabolism, these reported interactions have not been studied on the level of the isolated perfused liver.

3. Duration of Study

The techniques described in this proposal are presently being used in our laboratories. Thus, we anticipate that the proposed studies could be completed in two years.

4. Estimated Costs

a.	Personnel	
	1 full-time postdoctoral Research Associate	$17,000
	1 part-time laboratory assistant (animal care, lab maintenance, etc.)	5,000
	1 month of summer salary and benefits for P.I. and Co-Investigator	7,500
b.	Supplies and Chemicals: glassware, perfusion supplies, consumables and chromatographic columns	3,000
c.	Equipment maintenance and consumables for operation of instruments	4,000
d.	Animals	1,800
e.	Miscellaneous: office supplies, mail, travel, telephone, publications, etc.	2,000
	Total estimated budget (first year)	$40,300